

Optimization of Culture Conditions to Enhance Biosurfactant Production by Bacteria Isolated from Manyspiked Flatsedge (*Cyperus polystachyos*) Rhizosphere Soil

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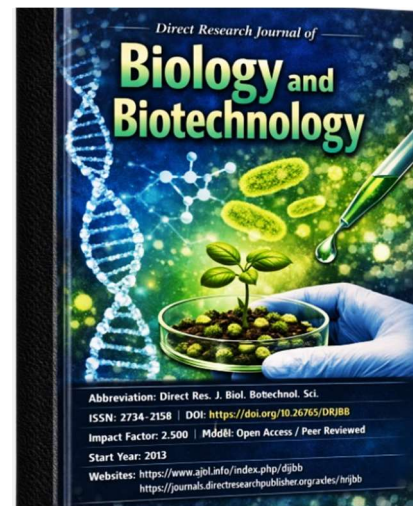
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ABSTRACT

This study explored the emulsification capabilities and optimization of culture conditions of bacterial isolates obtained from the rhizosphere soil of *Cyperus polystachyos* (Manyspike Flatsedge), emphasizing their potential for biotechnological applications. Soil samples were subjected to detailed physicochemical analysis to determine pH, electrical conductivity, and concentrations of nutrients and metals. The soil exhibited a slightly acidic pH (5.90) and moderate electrical conductivity (328 $\mu\text{S}/\text{cm}$). Nutrient analysis revealed nitrate (7.290mg/kg) and phosphate (1.417mg/kg) availability, alongside the presence of heavy metals such as iron (115.352mg/kg), zinc (12.313 mg/kg), chromium (5.112 mg/kg), aluminum (2.456 mg/kg), and nickel (17.472 mg/kg). A total of seventeen bacterial isolates were obtained and subjected to a series of biochemical tests and Gram staining. Preliminary screening for biosurfactant activity using emulsification index (E_{24}) revealed that *Klebsiella* sp. exhibited the highest activity, achieving an E_{24} index of 51.6%, followed closely by *Acinetobacter* sp. with an E_{24} index of 50%, *Bacillus* sp (E_{24} : 20 %), *Pseudomonas* sp (E_{24} : 5.9 %; 16 %), *Staphylococcus* sp (E_{24} : 0 %; 24 %) and *Micrococcus* sp (E_{24} : 14.2 %) suggesting their strong potential for biosurfactant production. Optimization of physicochemical parameters showed that all isolates exhibited poor or no biosurfactant production across tested pH levels (3, 5, 7, and 9), except *Pseudomonas* sp, which displayed positive biosurfactant activity at pH 5 and 7, suggesting moderate tolerance to slightly acidic and neutral conditions. Similarly, salinity tolerance tests (1%, 4%, 8%, and 10% NaCl) indicated that all isolates were inhibited under saline conditions, showing no biosurfactant activity. The emulsification activity demonstrated by *Klebsiella* sp. and *Acinetobacter* sp. highlights their applicability in bioremediation of hydrocarbon-contaminated sites and industrial processes requiring eco-friendly surfactants. The ability of *Pseudomonas* sp to produce biosurfactant at pH 5 and 7 suggest production can be optimized at such pH. Further investigations into optimizing growth conditions and characterizing biosurfactant properties are warranted to maximize their practical utility.

Keywords: Biosurfactant, Bioremediation, *Cyperus polystachyos*, Emulsification index, Optimization, Rhizosphere



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INTRODUCTION

The rhizosphere, a region surrounding plant roots, is a complex ecosystem comprising diverse microorganisms crucial for plant growth and soil fertility. Rhizosphere bacteria contribute to nutrient cycling, solubilize minerals, produce plant growth regulators, and suppress plant pathogens (Liu *et al.*, 2023). This study focuses on the

emulsification potential of bacteria isolated from *Cyperus polystachyos* rhizosphere soil. Emulsifiers are surface-active agents facilitating mixing of immiscible liquids, with applications in food, pharmaceuticals, and petroleum. Microbial emulsifiers offer advantages over chemical counterparts, including biodegradability and low toxicity.

Biosurfactants, a subclass of emulsifiers, have been isolated from bacteria, yeast, and fungi. *Cyperus polystachyos*, a tropical sedge adapted to diverse environments, holds potential for uncovering novel emulsifier-producing bacteria (Kumar *et al.*, 2020). This plant species is widely distributed across tropical regions and has been used in traditional medicine. Despite its potential, *Cyperus polystachyos*'s rhizosphere microbiome remains largely unexplored. Rhizosphere bacteria produce bioactive compounds, including antibiotics, antifungals, and emulsifiers (Cheng *et al.*, 2023). These compounds have applications in agriculture, pharmaceuticals, and cosmetics. Microbial emulsifiers exhibit diverse structures and properties, making them suitable for various industrial applications. The increasing demand for eco-friendly alternatives drives research toward exploring novel microbial sources. A significant knowledge gap persists regarding rhizosphere soil bacterial diversity and the emulsification potential of *Cyperus polystachyos* rhizosphere bacteria.

Biosurfactants are amphiphilic, low-molecular-weight compounds produced by microorganisms that possess the ability to reduce surface and interfacial tensions of liquids. Their dual nature comprising both hydrophobic and hydrophilic domains allow them to form micelles and emulsify hydrocarbons and other hydrophobic compounds (Chandankere *et al.*, 2021). These properties make biosurfactants valuable alternatives to synthetic surfactants in environmental, agricultural, and industrial applications due to their biodegradability, low toxicity, and high functional stability across a range of pH, salinity, and temperature conditions (Rufino *et al.*, 2021).

In recent years, there has been increasing global interest in identifying sustainable biosurfactant production strategies that do not depend on expensive substrates or genetically engineered strains. This interest has led to the exploration of indigenous microorganisms, microbes naturally present in polluted soils, saline waters, and extreme ecological niches for their innate capacity to produce biosurfactants under stressful conditions. These organisms have evolved biochemical mechanisms to adapt to local environments, often by secreting surface-active agents that increase the bioavailability of hydrophobic nutrients (Mubarak & Sattar, 2022, Ravikumar *et al.*, 2021, Singh & Kumar, 2022).

The screening and identification of biosurfactant-producing indigenous microbes typically involve isolation from contaminated ecosystems (e.g., oil spill sites, industrial waste zones), followed by biochemical assays like oil displacement, drop collapse, and hemolytic activity. Molecular characterization using 16S rRNA sequencing and metabolic profiling further confirms the biosurfactant-producing potential of these isolates (Awasthi *et al.*, 2023). The increasing global demand for environmentally friendly and sustainable bioproducts has drawn attention to biosurfactants as green alternatives to synthetic surfactants. Synthetic surfactants, though widely used, are

often toxic, poorly biodegradable, and harmful to ecosystems. Conversely, biosurfactants exhibit high biodegradability, lower toxicity, and functionality under extreme environmental conditions, making them attractive in applications such as oil spill remediation, heavy metal removal, food preservation, antimicrobial therapy, and enhanced oil recovery (Mukherjee *et al.*, 2021).

Microorganisms isolated from plant rhizospheres, particularly sedges like *Cyperus polystachyos*, are metabolically diverse due to their exposure to complex root exudates. These exudates, consisting of sugars, amino acids, and organic acids, provide selective pressures that promote microbial adaptation and secondary metabolite production, including biosurfactants. Previous studies have demonstrated that rhizosphere soils harbor *Pseudomonas*, *Bacillus*, *Serratia*, and *Acinetobacter* species, which are among the most efficient biosurfactant producers (Sharma & Pandey, 2020).

However, the commercial exploitation of biosurfactants remains limited by low yield and high production costs. Thus, optimization of growth conditions is critical to maximize biosurfactant output. Parameters such as pH, temperature, agitation, aeration, carbon and nitrogen sources, and incubation time significantly influence microbial metabolism and product synthesis (Banat *et al.*, 2021). By systematically optimizing these variables, indigenous bacteria can be harnessed for high-yield biosurfactant production.

This study is particularly significant for Nigeria, where *Cyperus Polystachyos* is widely cultivated for its edible tubers (commonly called tiger nuts). Its rhizosphere soils are often under explored but may harbor novel biosurfactant-producing bacteria with potential applications in local industries, environmental sustainability, and biotechnology. Despite the well-documented advantages of biosurfactant over synthetic surfactants such as biodegradability, lower toxicity, and functional stability under extreme environmental conditions their widespread industrial application remains limited. The major barrier to commercialization is the relatively low yield and high cost of biosurfactant production, which makes them less competitive compared to synthetic counterparts (Banat *et al.*, 2021).

Microorganisms such as *Pseudomonas* and *Bacillus* are well-known biosurfactant producers, yet their performance is highly dependent on growth conditions, nutrient availability, and environmental parameters. In many cases, suboptimal culture conditions result in poor biosurfactant output, limiting their industrial viability. Furthermore, most studies have focused on standard microbial strains, while indigenous bacteria from unique ecological niches such as rhizosphere soils remain under explored, especially in Nigeria.

The rhizosphere of *Cyperus polystachyos* (many-spiked flat sedge) represents a rich but untapped microbial reservoir. This plant's root exudates provide diverse nutrients that support microbial communities capable of

producing valuable metabolites, including biosurfactant. However, little or no research has been conducted on isolating and optimizing biosurfactant-producing bacteria from this specific rhizosphere soil in Nigeria. As a result, the potential of these indigenous microorganisms to serve as low-cost, eco-friendly biosurfactant sources has not been fully investigated. Additionally, without optimization of critical parameters such as pH, temperature, carbon and nitrogen sources, aeration, and incubation time, biosurfactant yields remain too low for practical applications in areas such as oil spill remediation, agriculture, food processing, and biomedicine. This knowledge gap poses a significant challenge to developing sustainable, locally adaptable biosurfactant production systems. The research objectives include (i) isolation and identification of biosurfactant-producing bacteria from the rhizosphere soil of *Cyperus polystachyos* (ii) screening microbial isolates for biosurfactant production using standard qualitative and quantitative assays. (iii) optimization of culture parameters such as pH, salinity and incubation time for maximum biosurfactant yield.

MATERIALS AND METHODS

Sample Collection

The sample collection was carried out along Bodo City, Ogoni land Rivers State. Mapped location for the area were; Soil sample with a latitude of 4°37'29" N, a longitude of 7°16'3" E. It was collected from an oil contaminated site

Soil Sample Dilution

Normal saline solution was utilized for serial dilution of the soil sample and the test was carried out as described by Sarin (2023). The solution of normal saline was prepared by dissolving 0.85 g of sodium chloride in 100 ml of distilled water. Subsequently, 9 ml of normal saline was added to labeled test tubes, each denoting specific dilution levels (10^{-2} to 10^{-3}) for the soil samples. Following autoclaving at 15 psi at 121°C for 15 minutes, 1g of soil was added to the 10^{-1} test tube and mixed gently. Serial dilution was performed through successive transfers with gentle agitation until the 10^{-4} test tubes were obtained.

Physiochemical Tests

The physiochemical analysis was carried out using the method adopted from Xiaojun (2020). The various tests are as follows: pH Measurement, Electrical Conductivity (EC) Test, Nitrate Test, Phosphate Test, Iron Test, Zinc Test, Chromium Test, Aluminum Test, and Nickel Test.

Isolation of Total Heterotrophic Bacteria (THB)

Adopting the method of Sneha (2023) and using the

spread plate technique, 0.1 ml aliquots of the 10^{-2} and 10^{-3} dilutions were inoculated in duplicate onto Nutrient agar plates. Plates were then incubated at 37°C for 24 hours after which the colonies were counted and subculture.

Isolation of Total Hydrocarbon Utilizing Bacteria (THUB)

The population of total hydrocarbon utilizing bacteria was estimated using the Vapor-phase method adopted from the description of Chikere (2023). Soil suspensions were plated on Mineral Salt Medium, and plates were sealed with sterile filter paper saturated with crude oil. Following incubation at 35-37°C for 3-5 days, colonies were counted to assess THUB populations.

Identification of Isolates

The identification of plant growth-promoting bacterial isolates was carried out using a combination of morphological, Gram staining, and biochemical characterization methods to determine their taxonomic position and physiological behavior. These tests are essential in distinguishing bacterial species based on colony appearance, cell wall composition, and enzymatic activities that reflect their metabolic versatility. The procedures adopted in this study were performed according to standard microbiological methods as outlined by Cappuccino and Sherman (2019); Cheesbrough (2021).

Biochemical Test for Identification of Bacterial Isolates

All procedures for the biochemical tests on the sample were carried out according to the methods described by Peter (2020). Biochemical tests are laboratory procedures used to identify microorganisms based on their chemical reactions and metabolic activities. These tests detect the ability of bacteria to produce enzymes, utilize substrates, or generate specific end products such as acids, gases, or alcohols. The different types of biochemical tests are ; Citrate Test, Indole Test, Catalase Reaction, Methyl Red-Voges Proskauer (MRVP) Test, Methyl Red Test, Voges Proskauer Test, Triple Sugar Iron Agar Test, Carbohydrate Fermentation, and Starch Hydrolysis Test.

Biosurfactant and Emulsification Activity

Biosurfactant production was done by preparing a culture medium composed of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 g/L), KH_2PO_4 (1g/L), NaNO_3 (3 g/L) and peptone (0.3 g/L) extract. The method described by Ali (2021) was adopted. Then, 9 ml of the medium was dispensed into test tube, sterilized and allowed to cool. Each tube was labelled with appropriate bacterial isolates code and inoculated with the isolates. 0.9 ml of diesel was added to each tube. The tube was incubated under stirring condition for 7 days.

Diesel was used as carbon source. The diesel was used in biosurfactant production or bio-emulsifier because their carbon chains are less complex. The specific medium for biosurfactant production was filtered and assessed as the water in oil (W/O) type, emulsification. 4ml of the filtered W/O from each tube was centrifuged at 4000 rpm for 15 minutes. 2 ml of cell free supernatant and 1ml of kerosene were added to the test tube and vortexed for 2min. The emulsification index (E_{24}), emulsion layer was measured immediately. The emulsion stability was checked after 24 hrs. E_{24} was defined as the percentage of the height of the emulsified layer divided by the total height of the liquid Column.

$$E_{24} = \frac{\text{Height of the emulsion layer}}{\text{Total height of liquid}} \times 100$$

Optimization of the biosurfactant produced

Factors used for optimization are: pH: at 3,5,7,9 and Salinity: 1%, 4%, 8%, and 10%. Base on the most active organisms; Organisms that were able to produce biosurfactant. A solution of Mineral Salt broth was prepared and the organism isolate is inoculated into the broth after sterilization based of the most active organism. For Salinity, 0.1g of NaCl is added to 10 ml of distilled water, it was dispersed into test tubes and sterilized at the optimal temperature. After sterilization, 1ml of the broth is inoculated into each of the test tubes. Then 0.5 ml of kerosene is added and incubated. For PH, dissolve the salts in distilled water, using a pH meter to measure the starting pH then gently adjust the pH either by adding NaOH to raise the pH (alkalinity) or by adding H_2SO_4 to reduce the pH (acidity) and sterilize. Afterwards, 1ml of the broth is inoculated into each of the test tubes and 0.5 ml of kerosene is added to it then incubated.

After 7 days, the test tubes are centrifuged for 20 minutes then each of the test tubes were vortexes for 2min and let to sit for 24 h.

RESULTS AND DISCUSSION

The emulsification potential of bacteria isolated from *Cyperus polystachyos* rhizosphere soil provides critical insights into the metabolic capabilities of microbial species in stressed environments. The physiochemical properties of the soil (Tables 1 and 2) indicate an acidic pH (5.90), low levels of key nutrients such as nitrate (7.290 mg/kg) and phosphate (1.417 mg/kg), and elevated concentrations of heavy metals, including iron (115.352 mg/kg) and nickel (17.472 mg/kg). These conditions create a selective pressure on microbial communities, favoring species capable of producing biosurfactants as a survival strategy. Such biosurfactants reduce surface tension and increase the bioavailability of hydrophobic compounds, making them highly desirable in biotechnological applications. The bacterial isolates tested in this study

Table 1: Physicochemical Analysis of the Sample.

Parameter	Result
pH	5.90
Electrical conductivity ($\mu S/cm$)	328
Nitrate (mg/kg)	7.290
Phosphate (mg/kg)	1.417
Iron (mg/kg)	115.352
Zinc (mg/kg)	12.313
Chromium (mg/kg)	5.112
Aluminium (mg/kg)	2.456
Nickel (mg/kg)	17.472

showed a wide range of emulsification potential (Table 3), with *Klebsiella* sp. (E_{24} = 51.6 %) and *Acinetobacter* sp. (E_{24} = 50 %) standing out as the most promising candidates. Both species showed a high yield having E_{24} greater than forty percent (40 %). In contrast, *Serratia* sp., *Proteus vulgaris*, and *Enterobacter* sp. exhibited no emulsification activity (E_{24} = 0 %), while two different *Pseudomonas* sp. recorded (E_{24} = 5.9 %; 16 %), two *Staphylococcus* sp. also recorded (E_{24} = 0 %; 24 %) and *Micrococcus* sp. showed moderate activity (E_{24} = 14.2 %). The high emulsification potential of *Klebsiella* sp. aligns with findings by Lal *et al.* (2022), who reported that *Klebsiella* sp. is a robust producer of glycolipid biosurfactants, particularly under nutrient-limited conditions. Glycolipids stabilize oil-water emulsions by reducing surface tension, enabling these bacteria to thrive in hydrophobic and stressed environments. Similarly, *Acinetobacter* sp., which achieved an E_{24} value of 50% in this study, has been widely documented for its ability to produce emulsan, a polysaccharide-lipid complex with exceptional emulsification properties (Das *et al.*, 2021). Both genera are commonly found in soil environments with high metal content, and their biosurfactant production is often upregulated as a response to environmental stressors, including heavy metals and low pH levels (Chakraborty *et al.*, 2021). The acidic nature of the rhizosphere in this study likely served as a trigger for these microbes to produce biosurfactants to maintain stability in an otherwise challenging environment. Interestingly, the lack of emulsification activity in isolates such as *Serratia* sp., *Proteus vulgaris*, and *Enterobacter* sp. (E_{24} = 0%) contrasts with some earlier studies. For example, Chakraborty *et al.* (2021) reported that *Serratia* sp. could produce rhamnolipids with moderate emulsification potential, but only under optimized laboratory conditions. This discrepancy could be due to the inhibitory effects of specific soil properties in the current study, such as heavy metal toxicity or the acidic pH, which might suppress the metabolic pathways required for biosurfactant production. Similarly, *Enterobacter* sp., previously shown to produce lipopeptides in controlled environments (Zhao *et al.*, 2023), exhibited no emulsification potential in this study. This suggests that the environmental conditions in the rhizosphere may not have been conducive to biosurfactant synthesis in these genera, emphasizing the importance of

Table 2: Morphological characteristics and Biological Test.

Isolate ID	Shape	Colour	Elevation	Edge	Size (mm)	Surface	Opacity	Catalase	Citrate	Glucose	Lactose	Indole	IMR	VP	Starch	Motility	TSIA Butt	TSIA Slant	H ₂ S	Gas	Gram Staining	Tentative Genera
JO1	Circular	Cream	Flat	Serrated	7	Smooth	Opaque	+	–	+	–	–	+	+	+	+	–	A	B	–	–	– rod
JO2	Circular	Yellow	Raised	Entire	1	Shinning	Opaque	+	+	+	–	–	–	–	–	–	–	B	B	–	–	+ cocci
JO3	Circular	Brown	Raised	Entire	3	Shinning	Opaque	+	–	+	+	–	+	+	+	+	+	A	A	–	–	+ cocci
JO4	Circular	White	Flat	Entire	3	Smooth	Translucent	+	–	–	–	–	+	+	–	–	–	A	B	–	–	– rod
JO5	Irregular	Cream	Raised	Serrated	5	Shinning	Translucent	+	+	+	–	–	+	–	–	+	+	A	B	–	+	+ cocci
JO6	Irregular	Cream	Raised	Serrated	6	Smooth	Translucent	+	+	+	–	–	+	–	–	+	+	A	A	–	+	– rod
PA1	Circular	White	Flat	Entire	5	Rough	Opaque	+	+	+	+	–	–	+	–	–	–	A	B	–	–	+ rod
PA2	Circular	White	Flat	Entire	1	Smooth	Opaque	+	+	–	–	–	+	+	–	–	–	A	B	–	–	– rod
PA3	Irregular	White	Flat	Serrated	6	Smooth	Opaque	+	+	+	–	+	–	–	–	–	+	B	B	–	–	– rod
PA4	Circular	Brown	Raised	Entire	3	Shinning	Opaque	+	–	+	+	–	+	+	+	+	+	A	A	–	–	– rod
PA5	Circular	White	Flat	Entire	1	Smooth	Opaque	+	+	–	–	–	+	+	–	–	–	A	B	–	–	– rod

Table 3: Emulsification Potential.

Bacterial isolate	Height (mm)	Total Height (mm)	E24 (%)
<i>Serratia</i> sp.	0.0	3.1	0.0
<i>Micrococcus</i> sp.	0.5	3.5	14.2
<i>Staphylococcus</i> sp.	0.0	3.0	0.0
<i>Acinetobacter</i> sp.	1.5	3.0	50.0
<i>Enterobacter</i> sp.	0.0	3.2	0.0
<i>Klebsiella</i> sp.	1.6	3.1	51.6
<i>Bacillus</i> sp.	0.5	2.5	20.0
<i>Pseudomonas</i> sp.	0.2	3.4	5.9
<i>Proteus vulgaris</i>	0.0	0.0	0.0
<i>Staphylococcus</i> sp.	0.6	2.5	24.0
<i>Pseudomonas</i> sp.	0.4	2.5	16.0

environmental triggers in activating biosurfactant production pathways. The moderate activity of *Micrococcus* sp. ($E_{24} = 14.2\%$) provides a middle ground in terms of emulsification potential. While lower than *Klebsiella* sp. and *Acinetobacter* sp., the activity of *Micrococcus* sp. may indicate a limited ability to produce biosurfactants under nutrient-limited conditions. Lal *et al.* (2022) noted that *Micrococcus* species tend to produce lower quantities of biosurfactants compared to other genera, often requiring the presence of specific substrates such as hydrophobic hydrocarbons or

carbon-rich media to maximize their biosynthetic pathways. This aligns with the current findings, where *Micrococcus* sp. showed only partial emulsification ability, possibly due to suboptimal environmental conditions in the rhizosphere. Comparing these results with previous studies highlights the ecological adaptability and metabolic diversity of *Klebsiella* sp. and *Acinetobacter* sp., which consistently perform well in stressed environments. Das *et al.* (2021) emphasized the versatility of these species in utilizing a wide range of carbon sources and their ability to produce high-

quality biosurfactants even under stress, such as heavy metal contamination and nutrient scarcity. On the other hand, the poor performance of *Serratia* sp. and *Staphylococcus* sp. suggests that biosurfactant production is strain-specific and may not occur universally within a genus, particularly in challenging soil environments. The findings of this study underscore the importance of soil physiochemical properties in shaping microbial metabolic capabilities. The high concentrations of iron and nickel in the soil may act as co-factors for enzymatic systems in *Klebsiella* sp.

Table 4: Emulsification of pH and Salinity after 24 hours

Sample code	pH	HE1	HM1	E24%(pH)	SA	HE2	HM2	E24% (SA)
PA1	pH3	0	3	0	1%	0	2.9	0
	pH5	0	3	0	4%	0	3.0	0
	pH7	0	3.2	0	8%	0	3.0	0
	pH9	0	3.2	0	10%	0	3.0	0
PA5	pH3	0	3.0	0	1%	0	3.0	0
	pH5	0	3.2	0	4%	0	2.6	0
	pH7	0	3.2	0	8%	0	2.4	0
	pH9	0	3.2	0	10%	0	3.0	0
PA10	pH3	0	3.0	0	1%	0	3.0	0
	pH5	0	3.0	0	4%	0	3.0	0
	pH7	0	3.0	0	8%	0	3.0	0
	pH9	0	3.0	0	10%	0	3.0	0
PA11	pH3	0	3.0	0	1%	0	3.0	0
	pH5	0.5	2.6	19.2	4%	0	3.0	0
	pH7	0.2	3.0	5.7	8%	0	3.0	0
	pH9	0	3.0	0	10%	0	3.0	0

Key: HE1 = Height of emulsion (pH); HM1 = Total height of mixture (pH); SA = Salinity; E24 % (pH) = Emulsification index (pH); HE2 = Height of emulsion (salinity); HM2 = Total height of mixture (salinity); E24 % (SA) = Emulsification index

and *Acinetobacter* sp., facilitating biosurfactant synthesis while potentially inhibiting the growth or activity of less resilient species (Zhao *et al.*, 2023). Moreover, the low pH and limited nutrient availability likely create a competitive environment that favors microbes capable of producing secondary metabolites, such as biosurfactants, to gain a survival advantage. Hence, this study reveals the significant emulsification potential of *Klebsiella* sp. and *Acinetobacter* sp. compared to other isolates from the rhizosphere of *Cyperus polystachyos*. These results are consistent with previous research emphasizing the ability of these genera to produce effective biosurfactants under stress. However, the variability in emulsification activity among isolates highlights the influence of environmental factors and strain-specific traits on biosurfactant production. Future studies could explore the optimization of growth conditions for less active isolates and investigate the genetic mechanisms underlying their biosurfactant production pathways to enhance their industrial and environmental applications.

The effect of pH on the emulsification potential of the isolates shows *Pseudomonas* spp. responded positively to pH variation, producing biosurfactants at pH 5 and pH 7 (Table 4). This suggests that *Pseudomonas* possesses biosynthetic pathways that are active under mildly acidic to neutral conditions. All other isolates failed to produce biosurfactants under the tested pH values, indicating narrow tolerance ranges or suppression of biosurfactant genes outside their natural pH. Table 4 confirms these observations, with *Pseudomonas* achieving the highest optimized emulsification index at pH 5 (19.2 %), followed by a reduced activity at pH 7 (6.7 %). This finding is in align with the result of Kaur and Kaur (2021) who worked on screening, optimization and characterization of biosurfactant produced by *Pseudomonas* sp. While for the effect of Salinity, salt concentration had a strongly negative

effect on biosurfactant production. All isolates were negative at various salinity levels, indicating inhibition of biosurfactant synthesis or reduced metabolic activity under saline stress. This result suggests that the biosurfactant-producing isolates are better suited for freshwater or low-salt environments and may be inhibited in high-salinity conditions such as marine or brackish habitats.

Conclusion

Bacteria isolated from the rhizosphere of *Cyperus polystachyos*, particularly *Acinetobacter*, *Klebsiella*, and *Micrococcus* species, exhibit significant emulsification potential through the production of biosurfactants. In general, the results of this study shows that the rhizosphere soil contains a diverse community of bacteria with distinct cultural and biochemical characteristics. Only some of these organisms were able to produce biosurfactants, and their emulsification strengths varied widely. A few strains showed strong emulsifying ability, indicating that they release surface-active compounds capable of stabilizing oil water mixtures. The optimization experiments revealed that biosurfactant production is highly sensitive to environmental conditions. Only one strain responded positively to changes in pH, showing better activity in slightly acidic to neutral conditions, while none produced biosurfactants under increased salinity. This suggests that the biosurfactant-producing bacteria from this soil are best suited to low-salt environments and may require specific pH conditions for maximum activity. The findings confirm that the soil hosts bacteria with useful biosurfactant-producing potential, but their performance depends strongly on growth conditions. A few strains stand out as promising candidates for further development in bioremediation and other applications that require effective natural surfactants.

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